510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number:
K042762

B. Purpose for Submission:
To obtain clearance for the Pefakit® APC-R Factor V Leiden assay, a plasma-based functional assay for the determination of resistance to activated protein C caused by the factor V Leiden mutation.

C. Analyte:
Resistance to activated protein C (APC-R)

D. Type of Test:
Plasma-based functional clotting assay

E. Applicant:
Pentapharm Ltd.

F. Proprietary and Established Names:
Pefakit® APC-R Factor V Leiden

G. Regulatory Information:

1. Regulation section:
   21 CFR 864.7925
2. Classification:
   Class II
3. Product Code:
   GGW
4. Panel:
   81 Hematology

H. Intended Use:

1. Intended use(s):
   Pefakit® APC-R Factor V Leiden is a plasma based functional assay for the determination of resistance to activated protein C caused by the factor V Leiden mutation (FV:Q506).

2. Indication(s) for use:
   Pefakit® APC-R Factor V Leiden is a plasma based functional assay for the determination of resistance to activated protein C caused by the factor V Leiden mutation (FV:Q506) on automated and semi-automated blood coagulation analyzers using mechanical or optical detection principle.

3. Special condition for use statement(s):
   Not applicable

4. Special instrument Requirements:
   Not applicable

I. Device Description:

Pefakit® APC-R Factor V Leiden is an in vitro diagnostic test kit containing 3 vials each of the following 4 lyophilized plasmas:

R1: APC/RVV-V (+APC) Reagent (APC, RVV-V, Polybrene, Hepes, BSA)

R2: APC/RVV-V (-APC) Reagent (RVV-V, Polybrene, Hepes, BSA)

R3: PTA Reagent (Prothrombin Activator, EDTA, Hepes, BSA)

R4: Dilution Plasma (human plasma, processed)

For quality assurance/quality control the corresponding control kit Pefakit® APC-R Factor V Leiden Controls has to be used.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   (a) COATEST® APC™ RESISTANCE V/ COATEST® APC™ RESISTANCE VS

2. Predicate K number(s):
   (a) K963111

3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
	Pefakit® APC-R Factor V Leiden	**Coatest APC Resistance V/Coatest APC Resistance VS**
Sample Type:	Lypophilized citrated human plasma	Lypophilized citrated human plasma
Reagent components:	FV deficient plasma and polybrene	FV deficient plasma and polybrene

Differences		
Item	**Device**	**Predicate**
Reagent components:	Snake venom	Phospholipids

Stability: Plasma reagent at 15-25º C (on board, once opened or after reconstitution)	8 – 24 hours	6 - 8 hours
Other reagents at 15-25º C (on board, once opened or after reconstitution)	24 hours	1 week
Plasma reagent at 2 – 8º C (once opened or after reconstitution)	14 days	24 hours (3 mos. at -20ºC)
Interference: Lupus anticoagulant	No influence	Abnormal clotting time, possible misleading results

K. Standard/Guidance Document Referenced (if applicable):

L. Test Principle:

Pefakit® APC-R Factor V Leiden is a plasma-based functional clotting assay and differs from other functional APC resistance tests by acting specifically on the prothrombinase complex level. It is based on a factor V-dependent prothrombin activator isolated from snake venom. Robustness and specificity of the assay is enhanced by elimination of possible disturbing influences by factors upstream of the coagulation cascade and independency from calcium.

Sample plasma is prediluted in dilution plasma and incubated at 37° C with factor V activator. Coagulation is triggered by the addition of a FV-dependent prothrombin activator in the absence of calcium. The ratio of the clotting time in the presence of APC and the clotting time in the absence of APC is determined.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   - *a. Precision/Reproducibility:*

     The coefficient of variation (CV) for all factor V Leiden genotype groups (homozygous, heterozygous, wild-type) and for the analytical instruments tested so far (BCS®, CA-500, STA®-R) within the series and from day to day determined by in-house and external validation studies was below 6%.

     Two plasma samples genotyped with PCR, one factor V Leiden heterozygous and one normal wild-type, were tested in a series of 25 measurements on the same day on two different fully automated analytical systems (BCS®, CA-500). The CV of the ratios for the plasma genotypes on both instruments was below 5%.

In a further study on the STA®-R analyzer, a series if 10 tests on each of 5 consecutive days were done using the same factor V Leiden heterozygous and normal wild-type plasmas. The CV of the clotting times and the ratios within each day and over all 5 days was ≤ 5.0 for the negative control and < 3.0 for the heterozygous control.

The values obtained during the 5 days were within the following ranges:

	C1 FVL Negative Control			C2 FVL Heterozygous Control		
	+APC(s)	-APC(s)	Ratio	+APC(s)	-APC(s)	Ratio
min	132.9	24.2	5.3	39.2	25.4	1.5
max	166.3	25.7	6.7	42.6	27.7	1.6

b. Linearity/assay reportable range:
Not applicable

c. Traceability (controls, calibrators, or method):
Not applicable

d. Detection limit:
Not applicable

e. Analytical specificity:
Factor deficiency:
The influence of factor deficiencies was tested in spiking experiments, where deficient plasmas were supplemented with increasing concentrations (0%, 25%, 50%, 75%, 100%) of the factor for which they were deficient. +APC and –APC clotting times were determined on an Amelung KC-10 A™.
No significant influence on ratio or test sensitivity in case of fibrinogen, prothrombin, FVIII, FX, ATIII, protein C, or protein S deficiency was found up to 100%. A deficiency of FV (<50%) may lead to strongly elevated clotting times and thus may lead to loss of discrimination performance.

Factor excess:
The influence of factor excess was tested in spiking experiments, where normal plasmas were supplemented with increasing concentrations (100%, 200%, 300%, 400%, 500%) of factor.
No significant influence on ratio or test sensitivity in case of excess of fibrinogen, FVIII, ATIII, or TPFI up to 5 times the normal value.

Anticoagulants:
To demonstrate that unfractionated heparin (UFH), low molecular weight heparins (LMWH), and pentasaccharides (PES) are inhibited by the addition of polybrene to the reagents, control plasmas were spiked with increasing amounts of UFH (Liquemin, 0.0-3.0 IU/ml), LMWH (Fraxiparin, 0.0-3.0 IU/ml) and PED (Arixtra, 0.0-2.0 µg/ml). Up to a concentration of ≤2.0 IU/ml for UFH and LMWH and a concentration of ≤2.0 µg/ml for PES, +APC and –APC clotting times for both controls were not or only slightly increasing and the ratios were within the predefined acceptable range.
Hirudin and argatroban are not inhibited by polybrene and thus preclude proper measurement at concentrations as low as 0.5 µg/ml.

The presence of lupus anticoagulant antibodies does not influence this test because there is no use of phospholipids in an APTT system. Due to the test principle, oral anticoagulant therapy (with warfarin or marcoumar) does not influence test outcome.

The influence of patient treatment with aprotinin or protamine was tested by spiking control plasma with increasing concentrations of the treatments. The presence if aprotinin (which inhibits the APC used in this test) and protamine in the patient's blood can result in decreasing ratios which were outside the acceptable range.

The effect if direct thrombin inhibitors (DTI) such as hirudin or argatroban are not inhibited by polybrene. Hirudin in the patient plasma has a strong effect on clotting times and thus precludes proper discrimination of the different genotypes.

Additional interferences:
15 citrated blood samples were centrifuged and the plasmas tested with the Pefakit assay on a BCS® coagulation analyzer, once freshly after collection and again after storage in a freezer at -80° C for 3 weeks and rethawing (5 minutes at 37° C). Clotting times were measured for +APC and –APC reagent and the ratio calculated according to the instructions given in the box insert. No relevant differences were observed between clotting times and ratios measured with fresh plasma and with frozen and re-thawed plasma respectively.

In order to test for influence of hemolytic plasma on test performance, a freshly drawn blood sample was frozen at -80° C and rapidly thawed at 37° C. The sample was centrifuged for 20 minutes, which gave hemolyzed plasma to be used for the dilution series. To both the negative and heterozygous control, 0 – 5% hemolyzed plasma was added and the resulting samples tested using

the submitted device. The results showed no influence on clotting time and ratio even at the highest level of 5% hemolyzed plasma.

In order to test for influence of platelet residues in the test plasma a platelet-rich plasma (PRP) was prepared by centrifugation of a freshly drawn blood sample. The supernatant of this sample was used to prepare a dilution series of 0-100% with normal pool plasma used a negative control. It showed that there was no influence on clotting time and ratio even at the highest level of 100% PRP.

	wt	het	hom	NC	Total
Wild-type (normal)	137	0	0	0	137
FV Leiden het	0	92	0	0	92
FV Leiden hom	0	0	7	0	7
Total	137	92	7	0	236

Sensitivity: 100% Specificity: 100%

f. Assay cut-off:
Not applicable

2. Comparison studies:

a. Method comparison with predicate device:
The comparative studies were performed at two sites: The Clinical Institute for Medical and Chemical Laboratory Diagnostics (CIMCLD), University of Austria and Duke University Medical Center (DUMC).

Side by side comparison studies were conducted between Pefakit® APC-R Factor V Leiden and COATEST® APC™ Resistance V as the predicate device on the same analyzer (STA® R) with the same batches of test kits.

Study design:
The studies were divided into three phases: familiarization, precision study and main clinical study. During the familiarization phase the investigator and the technicians acquired experience with the test method and its adaptation to the analytical device. In this phase, the investigator did some blind studies with samples provided by Pentapharm Ltd (FV-L status known by Pentapharm only) on three subsequent days. Decision on continuation of the study was made based on the reported results.

The precision study was performed in order to determine the intra- (20 consecutive tests with identical samples) and inter-assay (tests performed with identical sample on 10 consecutive days) precision of the test method on the analytical device. Precision testing was

performed with control plasmas provided in the Pefakit® APC-R Factor V Leiden Controls kit.

The data obtained in the inter-assay testing were used to establish a confidence range (mean ±2SD) for the use of these controls during phase 3. In phase 3, citrated samples (3.8% or 3.2%) were analyzed with Pefakit® APC-R Factor V Leiden and the predicate device, COATEST® APC™ Resistance V.

The samples included the following:

- At least 100 healthy subjects (50 females, no oral contraceptives, 50 males) with wild-type factor V confirmed by the routine functional test used at the DUMC, for determination of the normal range
- 50-100 subjects with heterozygous FV Leiden mutation confirmed by PCR method
- 10-25 subjects with homozygous FV Leiden mutation confirmed by PCR method
- 5-10 patients with confirmed deficiency of protein S
- 5-10 patients with confirmed deficiency of protein C
- At least 10 patients with confirmed lupus anticoagulants
- At least 10 patients with F VIII > 150%
- 5-10 patients with PT20210G/A mutation, if available
- 10 pregnant women (preferably 2nd and 3rd trimenon)

The samples excluded from the test group had the following conditions:

- Treatment wit a direct thrombin inhibitor (DTI) during the last 2 days
- Treatment with UFH, LMWH, or PES leading to a blood concentration > 2 U/ml
- FV deficiency (FV<30%)

Treatment with oral anticoagulants (vitamin K antagonists) was expected to have no influence on both tests and was neither an inclusion nor an exclusion criterion. This condition applied for up to about ¾ of all samples tested.

All tests were done in duplicate, analyzing with the COATEST APC assay followed by the Pefakit APC-R assay. Both test series were separated by a rigorous washing step. The test runs were only started once the ratio and time values obtained with the quality controls fell into the confidence range as established in phase 2 of the study. The QC testing was repeated if a test series contained more than 25 samples.

Precision results of CIMCLD Study

Intra-assay precision - Pefakit						
	Control C1			Control C2		
	+APC	-APC	Ratio	+APC	-APC	Ratio
Mean (n=20)	145.35	26.03	5.58	37.69	27.74	1.37
SD	8.09	0.61	0.24	0.44	0.49	0.05
CV	5.57	2.34	4.32	1.16	1.76	3.50

Intra-assay precision - COATEST						
	Level 1			Level 2		
	+APC	-APC	Ratio	+APC	-APC	Ratio
Mean (n=20)	37.7	83.7	2.2	38.6	62.5	1.62
SD	0.3	1.9	0.1	0.2	0.4	0.01
CV	0.8	2.3	2.3	0.58	0.70	0.82

Inter-assay precision - Pefakit						
	Control C1			Control C2		
	+APC	-APC	Ratio	+APC	-APC	Ratio
Mean (n=10)	154.86	25.91	5.97	43.58	27.27	1.59
SD	10.82	0.90	0.35	2.15	1.42	0.07
CV	6.99	3.49	5.88	4.93	5.21	4.67

Inter-assay precision - COATEST						
	Level 1			Level 2		
	+APC	-APC	Ratio	+APC	-APC	Ratio
Mean (n=10)	37.7	88.7	2.4	39.2	66.4	1.69
SD	1.3	4.6	0.1	1.4	4.1	0.10
CV	3.3	5.2	3.8	3.60	6.21	5.76

b. Matrix comparison:
Not applicable

3. Clinical studies:
   a. Clinical sensitivity:
   The diagnostic sensitivity (defined as the percentage of patient plasmas having a heterozygous or homozygous factor V Leiden mutation correctly classified as positive for this mutation) in all in-house and external validation studies was 100%. The 100% sensitivity claim was confirmed by both clinical studies for the device.

b. Clinical specificity:

The diagnostic specificity (defined as the percentage of patient plasmas not having a factor V Leiden mutation correctly classified as negative for this mutation) in all in-house and external validation studies was 100%. The 100% specificity claim was confirmed by both studies.

c. Other clinical supportive data (when a and b are not applicable):

Stability:
The Pefakit® APC-R Factor V Leiden Controls were used to determine clotting times and ratios for stability verification. All tests were performed on an Amelung KC-10 A™ micro equipment.

Stability of lyophilized reagents:
In preliminary stability tests on the sample lots 1-4, the reagents and controls showed to be stable at 2-8° C for more than a year.

In stress tests, the lyophilized reagents were exposed to 37° C for up to 14 days. Samples of the test kit reagents, R1-R4, were reconstituted on the first and second day, on day 10, day 12 and day 14. Samples of the controls C1 and C2 were also reconstituted on the first and second day, on day 6, day 8 and day 12. The +APC and -APC clotting times were measured. The clotting times were slightly rising over this time period, but the ratio remained stable during 14 days for the test kit reagent and 8 days for the controls.

Stability of reconstituted reagents:
After reconstitution, the open reagent R1-R4 were placed on the analytical instrument (15-25° C) and were tested using freshly prepared controls immediately after reconstitution, at 2 hour intervals over an 8 hour period, and again after 24 h, 32 h, and 96 h. During this period, the clotting times for the +APC measurement especially for the normal wild-type control (C1) were slightly rising, but were still within predefined acceptable range. This rise of clotting times had no relevant influence on the ration and on the power of the test to discriminate the different FV Leiden genotypes. It was concluded that the reagents have an on-board stability of at least 24 hours.

Reconstituted samples of reagents and controls were frozen and stored at -20° C. After a storage time of 6 months, the reagents and controls were thawed and clotting times and rations for C1 and C2 were determined. Clotting times and ratios were within the predefined acceptable ranges. No shift was observed. It was

concluded that the device and its reagent are stable when frozen at -20° C for at least 6 months.

Reagent R1-R4 were reconstituted and stored at 4° C. Over a period of 32 days clotting times and ratios were determined all 3 days. During the first 15 days the ratio remained stable within the predefined acceptable range. Beginning with day 18, the ratio began to drop. It was concluded that the reconstituted reagents can be stored at 2-8° C for at least 14 days.

Stability of plasma samples:
Blood samples were tested immediately after blood collection and plasma preparation and again after 24 hours storage at room temperature. Only a minimal rise of both clotting times and ratios was observed. It was concluded that plasma samples can stay as long as 12 hours on the analytical instrument with no influence on the discrimination power of the test.

Long term stability:
Long term stability studies were performed according to written standard protocols on sample lots 1-4 and on the pilot lots 1-3. Three test and three control kits released by QC were transferred to a controlled cool storage room at +5 C. Clotting times were determined with these test and control kits after a storage period of 3, 6, 9, 12, 18, 24, 36, 48, and 60 months. The study is ongoing and the longest storage time analyzed so far is 24 months. The ratios measured with these kits were within the predefined ranges.

4. Clinical cut-off:
   Not applicable

5. Expected values/Reference range:
   Typical ratio ranges for PCR-genotyped patient plasmas on different devices are shown in the tables below:

KC-4/-10 A micro		
Genotype FV:Q506	n	Ratio range (min/max)
Negative	25	4.2 – 6.9
heterozygous	104	1.3 – 1.9
homozygous	15	1.0 – 1.1

BCS (Behring Coagulation System)		
Genotype FV:Q506	n	Ratio range (min/max)
Negative	69	4.0 – 5.5
heterozygous	108	1.4 – 2.2
homozygous	17	0.9 – 1.1

CA-500/540		
Genotype FV:Q506	n	Ratio range (min/max)
Negative	25	4.2 – 6.0
heterozygous	105	1.4 – 1.8
homozygous	15	1.0 – 1.1

ACL 900		
Genotype FV:Q506	n	Ratio range (min/max)
Negative	53	3.2 – 5.4
heterozygous	57	1.4 – 2.1
homozygous	14	0.9 – 1.2

N. Proposed Labeling:

The labeling is sufficient and satisfies the requirements of 21 CFR section 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.